FRUIT PEELS PROBIOTICS: EFFECT ON SOIL PARAMETERS, MICROGREEN GROWTH, NUTRITIONAL PROFILE AND COLON CANCER CELL LINE

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A. THESIS TITLE

FRUIT PEELS PROBIOTICS-EFFECT ON SOIL PARAMETERS, MICROGREEN GROWTH, NUTRITIONAL PROFILE, AND COLON CANCER CELL LINE

B. INTRODUCTION

India faces a severe food waste crisis, with nearly 40% of total production, about 68.8 million tonnes, lost annually (FAO, 2024). Fruit processing industries contribute significantly, wasting 30–50% of mangoes, citrus, and pomegranates, and 20% of bananas (Sharma P *et al*., 2023). Excessive use of chemical fertilizer use (55 million metric tonnes in 2019–2020) threatens soil health, increase the need for eco-friendly alternatives (Lahiri, A. *et al*., 2023). Fruit peel waste is gaining attention as a soil enhancer, enriching soil and reducing environmental hazard. This study explores the extraction of *Bacillus* species from fruit peels as a sustainable alternative to chemical fertilizers. *Bacillus* inoculants promote plant growth, enhance soil health, and support waste repurposing in a circular economy (Mushtaq, Z *et al*., 2024). Fenugreek and wheatgrass microgreens are chosen for their high nutritional value and were incorporated into instant soup, chutney powder, and health mixes to promote daily consumption. Additionally, wheatgrass microgreens were tested against colon cancer (HCT) cells, addressing colorectal cancer (CRC), the second leading cause of cancer-related deaths globally. With existing CRC treatments posing adverse effects, dietary interventions offer a promising alternative (Tamraz, M *et al*., 2024). This research bridges sustainable agriculture, nutrition, and health, promoting eco-friendly farming utilizing fruit waste is in effective way hence looking for sustainable environment.

C. SCOPE OF THE STUDY

This study explores innovative approaches to address food waste management challenges, soil nutrient depletion, and micronutrient deficiencies in India. The research aims to enhance soil fertility and improve plant nutrient uptake by repurposing food waste, particularly fruit peels, as soil enhancers. This contributes to sustainable agricultural practices and provides a practical solution to declining soil health, a major concern for farmers striving to achieve optimal crop yields and profitability.

The study further investigates the role of *Bacillus subtilis*, a plant growth-promoting rhizosphere microorganism isolated from fruit waste, in improving soil and plant health. Incorporating *Bacillus subtilis* into the soil will significantly enhance the nutrient content, total phenolics, chlorophyll, and antioxidant levels in microgreens such as fenugreek and wheatgrass. These enriched microgreens will also serve as a basis for producing instant powders that address micronutrient deficiencies in the population, promoting better public health.

Additionally, fabricating a real-time NPK sensor is a key component of this research, providing farmers with an efficient tool for immediate soil nutrient analysis. This technology aims to reduce dependency on time-consuming laboratory testing, enabling farmers to make timely decisions to optimize soil management, improve yields, and increase profitability. By integrating these approaches, the study has the potential to offer holistic solutions that address critical agricultural and nutritional challenges, contributing to a sustainable and healthier future for both farmers and consumers.

D. OBJECTIVES

The specific objectives of the study are

1. Identification and Characterization of the technological, proximate, antimicrobial, and antioxidant properties of selected fruit peels
2. Utilization of selected fruit peel for soil enhancement and evaluating the effect on the morphology of fenugreek and wheatgrass microgreens
3. Isolation of *Bacillus subtilis* from fruit peel as a soil enhancer and analysis of the growth characteristics of fenugreek and wheatgrass microgreens
4. Fabrication of a real-time farmer-centric portable NPK sensor for soil nutrient (NPK) analysis
5. Formulation and Standardization of Instant Mix Using Nutrient-Enriched Microgreens
6. *In vitro* cytotoxicity analysis of wheatgrass on colon cancer (HCT) cells

E. METHODOLOGY

At the initial stage of the study approval from the Institutional Human Ethical Committee (Approval Number: AUW/IHEC/FSN-21-22/XPD-30) and Plant

Authentication (Fenugreek: BSI/SRC/5/23/2022/Tech-534 and Wheatgrass: BSI/SRC/5/23/2022/Tech-535) was obtained from Botanical Survey of India. The 16S rDNA sequence was published in the NCBI Gene Bankit nucleotide sequence database under accession number OR064147.

Phase I- Identification and Characterization of Biowaste - Fruit Peels

i. Selection of Fruit Peel Waste

Pomegranate, orange, and sweet lime peels were selected based on the literature and market survey. According to the Food Waste Index (2024), pomegranate and citrus peels contribute 30–50% of total fruit peel waste. These fruits are widely used for juicing, leading to significant discarded peels. The present study aims to utilize these waste peels sustainably. Fresh peels were collected, rinsed to remove contaminants, and drained. They were shade-dried for 96 hours under a relative humidity of 60-74% and a temperature of 28-32 °C and made into fine powder, and sieved through a sieve pore size of 0.5 mm.

ii. Characterization of Selected Fruit Peel Powders

The characterization of the fruit peel powders was conducted, including the analysis of technological properties such as bulk density, swelling capacity, and water absorption capacity. Additionally, color analysis (Dias *et al*., 2020), quantitative antioxidant assessment (DPPH assay), qualitative antimicrobial activity (Saleem & Saeed, 2020), FTIR analysis, and proximate composition (AOAC) (ash, moisture, fat, pH, crude fiber, and vitamin C) were done.

Phase II- Selection and Cultivation of Microgreens with Selected Fruit Peels

i. Selection and Cultivation of Microgreens in Fruit Peel Solution

Fenugreek and wheatgrass microgreens were selected for their high nutritional value. Fruit peel powder solutions (pomegranate, orange, and sweet lime) were prepared as soil enhancer (1g/100 mL water) and compared with a control. Seeds (25g each) were soaked, sown in 15 cm pots, and treated with fruit peel solution on the 0^{th}, $5^{th,}$ and 10^{th} day of the cultivation period. Growth parameters were assessed on the 15^{th} day under 63%-70% humidity at 30°C-32°C.

ii. Nutrient Parameters and Growth Parameters of Selected Microgreens Cultivated in Fruit Peel Solution Incorporated Soil

The soil nutrient test, including electrical conductivity, pH, calcium carbonate, organic carbon, phosphorus, potassium, iron, manganese, zinc, copper, boron, nitrogen, phosphorus pentoxide, and potassium oxide, was conducted following the standard NMSA procedure. The Sprout height, microgreen height, and total yield of the selected microgreens were analysed for the three variations and the control group. The best fruit peel with maximum soil nutrient content, sprout height, height of the microgreens, and yield of microgreens was identified for further investigation

Phase III- Isolation and Authentication of *Bacillus Subtilis* (Probiotic) from Pomegranate Fruit Peel

i. Isolation, Identification of Probiotic (*Bacillus subtilis*) From Pomegranate Peel and Biochemical Test:

Fruit peel powders were serially diluted and cultured on Bacillus agar using the streak plate method, with Pomegranate Peel (PP) showing the highest bacterial density. The culture was sub cultured, centrifuged, lyophilized, and DNA was extracted for PCR amplification. Agarose gel electrophoresis confirmed DNA presence, size, and purity, followed by 16S rDNA sequencing and NCBI authentication. A phylogenetic tree identified the strain as *Bacillus subtilis*. Biochemical tests was done to (Starch, Gelatin, and Casein Hydrolysis) assess the enzymatic activity (Firdus *et al*., 2022).

Phase IV- Experimentation on Selected Microgreens with Pomegranate Fruit Peel Powder and *Bacillus Subtilis*

i. Cultivation, Physicochemical, Phytochemical, Nutrient, and Toxicity Analysis of Microgreens in Different Variations:

25 number of fenugreek and wheatgrass seeds were sown in three pots under different variations: Pomegranate Peel solution (PP), *Bacillus subtilis* from Pomegranate Peel (Bs. PP), and a control. Growth parameters (sprout height, microgreen height, and total yield) were analysed on the 12th day. In PP, 100 mL of pomegranate peel solution was added, while Bs. PP received 500 µl of *Bacillus subtilis* cells on the 0^{th}, 5^{th}, and 10^{th} days. *Bacillus subtilis* presence in soil was confirmed using

the Quadrant Streak Method on the 3rd and 30th days. Phytochemical screening, chlorophyll, antioxidant, and nutrient content analyses were conducted for PP, Bs. PP and control. Additionally, brine shrimp lethality assay test was performed for the Bs. PP variation for both microgreens to ensure the safety of consumption.

Phase V- Construction and Calibration of Soil NPK Nutrient Sensor

Components such as the Arduino microcontroller, NPK multiplexer, Node MCU, AS7265X spectrometer sensor, OLED display, electrodes, and connectors were procured for fabricating the soil NPK sensor. The Arduino Uno and Node MCU were programmed using Arduino software. The NPK values were recorded from untreated soil and used as a standard for further analysis. The sensor was then used to measure NPK levels in soil treated with pomegranate peel and *Bacillus subtilis* from pomegranate peel. Additional validation was conducted on random agricultural land, and farmers' video testimonials were recorded. Finally, the cost of the soil NPK sensor was calculated.

Phase VI- Formulation and Standardization of Instant Powders using Selected Microgreens

Phase IV results showed that the Bs. PP variation had the highest nutritional profile compared to PP and control. Based on this, instant chutney and soup mix powders were developed using Bs. PP-treated fenugreek and wheatgrass microgreens. Dried and powdered fenugreek and wheatgrass microgreens were incorporated into instant chutney and soup powders at 2g, 4g, 6g, and 8g ratios. Additional ingredients for Instant chutney powder included black gram dhal, Bengal gram dhal, cumin, garlic, dry chili, and salt, while instant soup powder contained cumin, garlic, onion, pepper, turmeric, tomato powder, and salt. Sensory evaluation and cost analysis were then conducted.

Phase VII- *In Vitro* Cytotoxicity Analysis of Wheatgrass on Colon Cancer (HCT) Cells

An in vitro cytotoxicity analysis of Bs. PP-treated wheatgrass on colon cancer (HCT) cells was conducted. Wheatgrass instant powders showed higher overall acceptability than fenugreek instant powders. MTT assay and apoptotic staining were performed to evaluate its cytotoxic effects.

F. ORIGINAL CONTRIBUTIONS

The salient findings of the study are as follows,

Phase I- Identification and Characterization of Biowaste - Fruit Peels

i. Characterization of Selected Fruit Peel Powders

The technological properties of fruit peels showed that pomegranate peel had the highest bulk density, swelling, and water absorption capacity, enhancing soil structure and moisture retention. Proximate analysis revealed that pomegranate peel had high crude fiber, fat, and vitamin C, while orange peel had the highest ash content, and sweet lime peel had the highest moisture content. Antioxidant analysis confirmed that pomegranate peel had the strongest inhibition, followed by orange and sweet lime peels. Antibacterial studies showed sweet lime peel was most effective against *S. aureus* (24 ± 1.0 mm), while pomegranate peel had the highest inhibition against *E. coli* (20.3 ± 0.6 mm). FTIR confirms the presence of water, alcohol, carboxylic acid, alkane, amines, aldehyde, anhydride, ester, ketone, amide, nitrogen dioxide, fluorine and chlorine

Phase II- Selection and Cultivation of Microgreens with Selected Fruit Peels

i. Nutrient Parameters and Growth Parameters of Selected Microgreens Cultivated in Fruit Peel Solution Incorporated Soil

Soil analysis showed that PP had the highest levels of essential nutrients, while OP had the highest organic carbon content. Growth studies indicated that PP significantly improved sprout height, microgreen height, and yield for both fenugreek and wheatgrass. Fenugreek microgreens with PP reached 2.9 cm in sprout height, 14.33 cm in height, with the highest yield (60.33 g), while wheatgrass sprout height (2.2 cm), height of microgreens (24.47 cm), and total height (24.47 cm) were also greatest under PP treatment. Tukey's post hoc test confirmed significant differences between variations, with PP showing the most beneficial effects. These findings suggest that pomegranate peel is the most effective treatment for enhancing soil nutrients, plant growth, and microgreen yield.

Phase III- Isolation and Authentication of *Bacillus Subtilis* (Probiotic) From Pomegranate Fruit Peel

i. Isolation, Identification of Probiotic (*Bacillus subtilis*) From Pomegranate Peel and Biochemical Test:

Agarose gel electrophoresis confirmed successful amplification of the 16S rRNA gene, showing a distinct DNA band at 909 bp. Sequencing identified the bacterium as *Bacillus subtilis*, with phylogenetic analysis using PHYLIP showing high similarity to NCBI BLAST sequences. The 16S rDNA sequence was published in the NCBI GenBank (OR064147). Biochemical tests for starch, gelatin, and casein hydrolysis yielded positive results, confirming *Bacillus subtilis*. Its enzymatic activity enhances microbial activity and nutrient availability.

Phase IV- Experimentation on Selected Microgreens with Pomegranate Fruit Peel Powder and *Bacillus Subtilis*

i. Cultivation, Physicochemical, Phytochemical, Nutrient, and Toxicity Analysis of Microgreens in Different Variations:

The height and yield of fenugreek and wheatgrass microgreens were assessed on the 12th day under *Bacillus subtilis* (Bs. PP) and Pomegranate Peel (PP) variations. Bs. PP showed the highest growth and yield, followed by PP and control. Tukey's test confirmed significant differences, highlighting Bs. PP's effectiveness in enhancing microgreens growth. The quadrant streak analysis was done to confirm the presence of *Bacillus subtilis* in soil on the 3rd and 30th day of the cultivation period. The qualitative phytochemical analysis of fenugreek and wheatgrass microgreens, treated with two variations, Pomegranate Peel (PP) and *Bacillus subtilis* isolated from Pomegranate Peel (Bs. PP), along with a control group using an aqueous extract, confirmed the presence of various bioactive compounds. These included flavonoids, alkaloids, sterols, anthraquinones, carbohydrates, and cardiac glycosides. Bs. PP had the highest chlorophyll content in both fenugreek (40.5 mg/l) and wheatgrass (47.83 mg/l), followed by PP and C. Bs. PP had the highest phenolic content in fenugreek (14.3 mg GAE/mL) and wheatgrass (27.45 mg GAE/mL), followed by PP and C. Bs. PP-treated fenugreek (2.33 mg) and wheatgrass (5.61 mg) microgreens had the highest antioxidant content, followed by PP and Control. *Bacillus subtilis'* microbial activity significantly enhanced antioxidant levels. Tukey's test confirmed significant differences,

with Bs. PP is the most effective. The results of the nutrient analysis show that B. PP-treated fenugreek microgreens had the highest protein, carbohydrates, iron, calcium, and folic acid and Bs. PP-treated wheatgrass microgreens had the highest levels of protein, carbohydrates, iron, sodium, and folic acid, while PP-treated wheatgrass had the highest levels of phosphorus and potassium. The toxicity study showed that Bs. PP was tested at various concentrations against a control (K_2 Cr_2 O_7) and a blank (saline water), showing low toxicity with a maximum mortality of 13% at 500 µg/ml in 24 hours. In wheatgrass microgreens, Bs. PP exhibited minimal toxicity, with a maximum mortality of 30% at 1500 µg/ml in 24 hours.

Phase V- Construction and Calibration of Soil NPK Nutrient Sensor

The soil NPK nutrient sensor system integrates multiple components for accurate soil analysis. The Arduino microcontroller acts as the central unit, connecting the AS7265X spectrometer for spectral analysis and an NPK multiplexer with electrodes to measure Nitrogen (N), Phosphorus (P), and Potassium (K) concentrations. NodeMCU enables wireless data transmission for real-time monitoring, while an OLED display provides on-site readings. The system operates by initializing and calibrating sensors, collecting NPK data, and processing it via the Arduino. Data is transmitted through serial communication to NodeMCU for remote access on mobile and laptop screens, while the OLED display presents values locally. Arduino software was used to program the system, configuring communication protocols, calibrating sensors, and ensuring precise soil analysis. Product cost calculation of soil NPK Nutrient sensor: The total cost of components, including overhead charges, is ₹2,000 stating that this cost effective NPK nutrient sensor can be used for local farmers

Phase VI- Formulation and Standardization of Instant Powders using Selected Microgreens

Sensory evaluation of fenugreek and wheatgrass instant chutney and soup powders was conducted using a 9-point hedonic scale with 30 trained judges. Control samples received the highest scores, while formulations with 2g–4g of fenugreek or wheatgrass maintained good acceptability. The overall acceptability of the fenugreek instant chutney powder (8.2 ± 0.63) was highest in variation FCP1 compared to all other variations. The overall acceptability of the fenugreek instant soup powder (7.5 ± 1.35) was highest in variation FSP1. The overall acceptability of the Wheatgrass instant

chutney powder (8.70 ± 0.50) was highest in variation WCP1 compared to all other variations. The overall acceptability of the wheatgrass instant soup powder (8.67 ± 0.48) was highest in variation WSP1 compared to all other variations. Cost analysis showed raw ingredients expenses of Rs. 12 for instant soup powder and Rs. 10 for instant chutney powder, with additional processing, electricity, packaging, and overhead costs totaling Rs. 27 and Rs. 25, respectively. Both products were priced at Rs. 30, yielding Rs. 3 profit for instant soup powder and Rs. 5 for instant chutney powder.

Phase VII- *In Vitro* Cytotoxicity Analysis of Wheatgrass on Colon Cancer (HCT) Cells

The MTT assay on wheatgrass-treated colon cancer (HCT 116) cells showed morphological changes like shrinkage, detachment, membrane blebbing, and distortion at higher concentrations. Apoptotic staining indicated live (green), early apoptotic (yellow), and late apoptotic (orange) cells due to chromatin condensation. Cell viability decreased as extract concentration increased, with IC_{50} values of 795.436 ± 15.271 µg/ml at 24 hours and 575.44 ± 37.984 µg/ml at 48 hours. The 48-hour treatment was more effective, requiring a lower concentration for 50% cell inhibition.

G. CONCLUSION

The findings of this study emphasize the significant potential of fruit peel soil enhancer, *Bacillus subtilis* inoculation, and real-time soil nutrient analysis in revolutionizing agricultural practices. The results indicate that fruit peels, particularly pomegranate peel, effectively enhance the growth of fenugreek and wheatgrass microgreens by providing essential nutrients and organic matter. Among the tested fruit peels, the most effective one was identified based on growth parameters such as plant height and yield.

Furthermore, the incorporation of *Bacillus subtilis* isolated from pomegranate peel demonstrates a substantial improvement in the nutritional profile of microgreens. By increasing total phenolic content, antioxidant activity, and chlorophyll levels, *Bacillus subtilis* enhances the nutritional value of microgreens, making them a viable option for addressing micronutrient deficiencies. This aspect of the study provides insights into microbial soil enhancer as a natural and sustainable alternative to chemical fertilizers, promoting healthier crop production while minimizing environmental impact.

The NPK Multiplexer Module Sensor System is used for soil NPK nutrient analysis, essential for sustainable agriculture. The sensor system was constructed by integrating various components, including an NPK sensor, AS7265X spectrometer, Node MCU, Arduino microcontroller, and an OLED display, all connected to ensure seamless operation. A unique feature of this system is the use of copper and aluminium electrodes, which create small electrical vibrations in the soil to enhance measurement precision. Wireless data transmission and remote monitoring capabilities further improve accessibility for farmers and researchers. To ensure accuracy in predictions, the system underwent more than 100 calibrations across different soil types, refining its performance. This extensive calibration process is crucial for future developments, paving the way for real-time NPK sensors with even higher accuracy. With its innovative approach, the system provides a cost-effective and efficient solution for optimizing soil health, marking a significant advancement in precision agriculture.

Overall, this research provides a comprehensive approach to sustainable agriculture by integrating fruit peel-based biofertilization, microbial enhancement of plant nutrition, and advanced soil analysis tools. These findings contribute towards improving food security, reducing food waste, and promoting environmentally friendly farming practices. By repurposing agricultural waste, enhancing soil microbiology, and leveraging technological advancements, this study paves the way for innovative and sustainable solutions in modern agriculture.

Moving forward, further research can explore scaling up these approaches for larger agricultural applications, optimizing formulations of fruit peel soil enhancer, and refining NPK sensor technology for wider accessibility. Collaboration with farmers, agricultural organizations, and policymakers will be essential in translating these findings into practical, real-world applications. The study's holistic approach to sustainable farming offers a promising path toward addressing critical agricultural and nutritional challenges, ensuring a healthier and more resilient food system for the future.

H. STRENGTH OF THE STUDY

1. The study demonstrated that fruit peels like pomegranate, orange, and sweet lime effectively enhance soil quality.
2. *Bacillus subtilis* from fruit peels serves as an excellent soil amendment, promoting microgreen growth and yield.

3. The developed NPK soil nutrient sensor aids in improving real-time accuracy for soil nutrient analysis.
4. Wheatgrass-enriched instant mixes boost nutritional intake and exhibit potential anticancer effects on HCT 116 cells.

I. LIMITATION OF THE STUDY

1. Ash content, moisture, pH, crude fiber, fat, and vitamin C were analyzed only for selected fruit peels.
2. The probiotic *Bacillus subtilis* was identified using culture on Bacillus agar medium, PCR amplification, agarose gel electrophoresis, and 16S rDNA sequencing, followed by phylogenetic tree analysis.
3. Only *Bacillus subtilis* isolated from pomegranate peel was used due to its ability to survive in dry soil.
4. The presence and persistence of *Bacillus subtilis* were tested solely using the quadrant streak method.

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